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A NIR spectroscopy-based efficient approach to detect fraudulent additions within mixtures of dried *porcini* mushrooms

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ABSTRACT

Boletus edulis and allied species (BEAS), known as "*porcini* mushrooms", represent almost the totality of wild mushrooms placed on the Italian market, both fresh and dehydrated. Furthermore, considerable amounts of these dried fungi are imported from China. The presence of *Tylopilus* spp. and other extraneous species (*i.e.*, species edible but not belonging to BEAS) within dried *porcini* mushrooms – mainly from those imported from China and sold in Italy – may represent an evaluable problem from a commercial point of view.

The purpose of the present study is to evaluate near-infrared spectroscopy (NIRS) as a rapid and effective alternative to classical methods for identifying extraneous species within dried *porcini* batches and detecting related commercial frauds.

To this goal, 80 dried fungi including BEAS, *Tylopilus* spp., and *Boletus violaceofuscus* were analysed by NIRS.

For each sample, 3 different parts of the pileus (pileipellis, flesh and hymenium) were analysed and a low-level strategy for data fusion, consisting of combining the signals obtained by the different parts before data processing, was applied.

Then, NIR spectra were used to develop reliable and efficient class-models using a novel method, partial least squares density modelling (PLS-DM), and the two most commonly used class-modelling techniques, UNEQ and SIMCA.

The results showed that NIR spectroscopy coupled with chemometric class-modelling technique can be suggested as an effective analytical strategy to check the authenticity of dried BEAS mushrooms.

1. Introduction

Out of all the wild mushrooms in the world, there are few as prised and sought after as *Boletus edulis* Bull. and allied/related species (BEAS) [1]. BEAS, commonly named *porcini*, stands for a set of fungal species including, in Europe, *Boletus edulis* Bull., *B. aereus* Bull., *B. aestivalis* (Paulet) Fr., and *B. pinophilus* Pilát & Dermek, and dozens of other species worldwide [2–5], among which several Chinese species recently described elsewhere [6]. BEAS belong to Section *Boletus* (ex Sect. *Edules* Fr.), which is today considered representative of all the species of genus *Boletus* L. s. str. [7] (*Boletaceae, Boletales, Basidiomycota*). The purplish-hued Asian species *Boletus violaceofuscus* W.F. Chiu can be considered controversial for its placement inside or outside the BEAS. During last decade *Boletus violaceofuscus* was considered, on a morphological basis, as belonging to the section *Boletus* [8]; then a molecular study by Mello et al. [9] showed that it clustered outside the section *Boletus*. Now it has been ascribed again within section *Boletus*, even if in a distinct lineage called *Alloboletus* by recent molecular revisions [2,6,10].

BEAS are among the edible mushrooms the most widely collected in the world [11]. Dentinger et al. [2] affirm that their economic value is clearly substantial since 20.000–100.000 metric tons are estimated to be consumed annually and the median wholesale price in the U.S. for fresh mushroom in 2009 was *ca.* US $60/kg and can reach US $200/kg. It is worth noting that also in Europe mushroom market is an important source of revenue for a number of rural regions areas [11]. In the Mediterranean area, BEAS are an essential component of the traditional culture and cuisine, especially in Italy [1].

A significant portion of *porcini* are dried, packaged and then distributed worldwide. However, most of the *porcini* available on

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the Italian market or exported by Italy are imported from Eastern Europe and China where they are collected, dried on site, and then subjected to a first selection.

Unfortunately, among imported fungi ascribed to BEAS there is the presence of different, less valuable fungi, some of them not edible or not marketable according to some national laws.

Analyses performed to define macrofungi eligible for sale are mainly based on naked eye inspection aimed at identifying extraneous species (species, edible or not, not belonging to BEAS) and/or macromorphologic alterations.

Several species of the genus *Tylopilus* can be intermixed with BEAS, especially in the batches of dried *porcini* imported from China [12]. Several of these Asian species belong to the *Tylopilus plumbeoviolaceus* complex and are notoriously difficult to individuate due to their morpho-chromatic and organoleptic affinities [12,13]. These species can be easily confused with BEAS by a trivial visual inspection, even if a simple taste test highlights an intense bitter flavour.

Identifying *Tylopilus* spp. intermixed with BEAS is very difficult, not only for workers employed in mushroom manual selection and packaging, but also for mycologists.

BEAS derived products may be adulterated even with *Boletus violaceofuscus*. From a commercial point of view, this species independently of molecular analysis, is quite distinct from European species of *porcini* for its purple basidiomata and it is not considered belonging to BEAS. Consequently, it represents an "extraneous species" in the dried *porcini* batches. Anyway, detection of the presence of small amounts of such a species among dried specimens could be not so straightforward.

Visual inspection of basidiomata performed by professional mycologists is the most adopted method for dried mushroom identification: up to now, no instrumental analytical techniques have been proposed to identify dried BEAS.

The present study describes an original, rapid, efficient and non-destructive analytical method, based on near infrared spectroscopy (NIRS) coupled with chemometrics, to detect additions of lower-quality and/or non-European mushroom species intermixed with BEAS.

To this goal, 80 dried fungi (44 BEAS, 20 *Tylopilus* spp. and 16 *Boletus violaceofuscus*) were analysed by NIRS.

The study was focused on the pileus (the technical name for the cap of a sporoma or fungal fruiting body), which is one of the most characterising anatomical portions. In more detail, for each fungus, three parts of the pileus were considered and analysed: pileipellis (cortical layer of pileus), flesh (layer under the pileipellis and above the hymenium), and hymenium (spore-bearing layer of the sporoma).

According to the classification proposed by Durrant-Whyte [14], a complementary data fusion was performed: "*the information provided by the input sources represents different parts of the scene and could thus be used to obtain more complete global information…*". In particular, in this study, a low-level fusion approach, consisting in combining the whole signals provided by the different fungus parts before data processing was tested.

Then, a class-modelling approach was followed, aimed at characterising the BEAS samples. In more detail, unequal dispersed classes (UNEQ) [15,16] and soft independent modelling of class analogy (SIMCA) [17,18], the class-modelling techniques most commonly applied in chemometrics, were used. Model performances, evaluated in terms of efficiency (geometric mean of sensitivity and specificity), indicated that data structure was quite complex and would have gained a benefit from the application of a method more capable to model non-normal distributions.

For this reason, a novel PLS-based class-modelling strategy was applied, called partial least squares density modelling (PLS-DM), which combines the features of PLS and potential function methods (PFM), together with *Q* statistics, to obtain highly efficient class models for the characterisation of BEAS fungi. This method was presented in 2014 by Oliveri et al. [19]. The efficiency of PLS-DM models, fully validated by means of an external test set, showed that NIR spectroscopy can be used as a valid tool for the verification of "authenticity" of BEAS.

2. Experimental

2.1. Samples

80 samples of dried mushrooms were analysed. They were samples of dried BEAS, *Tylopilus* spp. and *Boletus violaceofuscus*. Specimens were provided by different suppliers from different origins. In more detail, BEAS originated from Europe (Poland, Hungary, Bulgaria, Romany, Serbia, Macedonia, and other Balkans), Russia, and China (Yunnan province), whereas *Tylopilus* spp. and *Boletus violaceofuscus* were from China. All of the specimens were collected in three different years. Since the goal of this study is the characterisation of BEAS fungi, the 80 samples were divided into two classes:

1) 44 BEAS (the target class to be modelled);
2) 36 non-BEAS comprehending 20 *Tylopilus* spp. and 16 *Boletus violaceofuscus* (used to evaluate specificity of the BEAS class models).

2.2. Apparatus and procedure

2.2.1. NIR Spectroscopy

NIR measurements were performed by a FT near-infrared spectrometer, based on a polarisation interferometer (Buchi NIR-Flex N-500), in the 4000–10,000 cm^{-1} range with a 4 cm^{-1} resolution, and a total of 512 scans were averaged for every spectrum. The diameter of the circular surface analysed was reduced to 3.0 mm by using a specific adaptor.

2.3. Data analysis

One spectra for each part (pileipellis, flesh, and hymenium) of the sample was recorded; then a segment of the signals, from 9000 to 10,000 cm^{-1}, was removed because non-informative.

Thus three spectra were available for each fungus and three NIR data matrices having 80 rows (samples) and 1250 columns (variables, reflectance at different wavenumbers) were built. For simplicity, these data matrices will be referred to as: $\mathbf{P1}_{80,1250}$, $\mathbf{P2}_{80,1250,}$ and $\mathbf{P3}_{80,1250,}$ respectively for pileipellis, flesh and hymenium. **P1**, **P2**, and **P3** were submitted to standard normal variate (SNV) transform and second derivative; derivative spectra were calculated with a Savitzky–Golay filter using a third-order polynomial and an 11-point window.

In order to extract useful complementary information from the different parts of mushrooms analysed, a strategy for data fusion was applied and the three pre-treated NIR data matrices were combined, forming new data matrices. In particular, two unified matrices were tested:

1) $\mathbf{U1\text{-}2\text{-}3}_{80,\ 3750}$ obtained combining all the three data matrices **P1**, **P2** and **P3**.
2) $\mathbf{U1\text{-}3}_{80,\ 2500}$ obtained combining only the two mushroom parts found to be most informative, *i.e.*, pileipellis and hymenium.

As the first step, principal component analysis (PCA) [20] was applied on the three pre-treated separate matrices (**P1**, **P2**, and **P3**) and also on the unified matrices, as a display method in order to visualise data structure.